



## MINERAL ELEMENT CONTENT AND ANTIOXIDANT CAPACITY OF SOME LATVIAN BERRIES

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Berries are widely used nowadays in prevention and in adjuvant therapy of different diseases because of their valuable bioactive agents, antioxidant, anti-tumor and anti-inflammatory properties. It has been observed that berries are used very frequently but without medical control. The main aim of our study was to determine the element content and antioxidant activities in some Latvian varieties of berries (e.g. blueberry, *Vaccinium corymbosum* L., bilberry, *Vaccinium myrtillus* L. and red berry, *Vaccinium vitis-idaea* L.). Element content was measured by ICP-OES. Total antioxidant activity was determined by chemiluminometry and hydrogen-donating ability was measured by spectrophotometry. The berries under examination contain elements in relatively low concentrations and the consumption of these kind of berries is also poor, although they might be good sources for some essential elements; such as blueberry for Mo, bilberry for Li, Mn, Mo and red berry for Cr, Li, Mn, Mo. On the other hand, they have good antioxidant properties, especially bilberry. Beneficial antioxidant capacities and moderate metal ion concentrations support that berries can complete a diverse diet, and they may be a good supplement in some metal-accumulating disorders.

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### Introduction

Berries contain valuable bioactive agents as unsaturated fatty acids, vitamin C, phenolic compounds such as polyphenols and anthocyanins.<sup>1,2</sup> Berries are frequently used in prevention and in adjuvant therapy of different diseases in our time since they are known to have several favorable effects because of their antioxidant, antitumor and anti-inflammatory properties.<sup>3,4,5</sup>

Berry fruits as foods products are widely available in the market in various forms (fresh fruits, dried or frozen fruits, jam, syrup, different food supplements, etc.) and as products (extracts, supplements) in drugstores and pharmacies, therefore people can consume a relatively large amount without any control from medical authorities. Since berries can be consumed almost every day, therefore a study on their quantity of essential and toxic metal ions is essential other than the bioactive agents (flavonoids, organic acids, polyphenols), as well as antioxidant effects in vitro and the investigation of absorption (availability).

In the present article our aim was to evaluate the element content and antioxidant activities in some Latvian variety of berries.

### Experimental

#### Materials

Blueberry (*Vaccinium corymbosum* L.), bilberry (*Vaccinium myrtillus* L.) and red berry (*Vaccinium vitis-idaea* L. var. *vitis-idaea*) were collected in the forests near Riga or bought in from the local markets in Riga, Latvia.

Infuses were made by steeping of 5g berries in 50 mL water for a day at room temperature. After filtration the aqueous extracts were used for the antioxidant properties measurements.

Luminol, microperoxidase, 30% hydrogen peroxide and 2,2-diphenyl-1-picrylhydrazyl were obtained from Sigma-Aldrich (St. Luis, USA). Methanol and Na<sub>2</sub>CO<sub>3</sub> were obtained from Reanal-KER (Budapest, Hungary). Nitric acid originated from Carlo Elba by Reanal (Budapest, Hungary) and multielement ICP standard solution were bought from Molar Chemical (Budapest, Hungary).

#### Measurement of elements

Element concentrations (Al, As, B, Ba, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Ni, P, Pb, S, Si, Sn, Sr, Ti, V, Zn) in the berries were determined by inductively coupled plasma optical emission spectrometry (ICP-OES). Type of instrument: Spectro Genesis ICP-OES (Kleve, Germany). After digestion of the samples (0.5 g from drug) with a mixture of nitric acid and hydrogen peroxide (10 mL + 4 mL) and dilution with de-ionized water to 25 mL, concentrations of elements were determined.<sup>6</sup>

### Total scavenging capacity

A chemiluminescence assay adopted to a Berthold Lumat 9501 (Bad Wildbad, Germany) instrument applied for the determination of total scavenger capacity of the berries by the method of Blázovics et al.<sup>7</sup> The reaction mixture for standard background consisted of hydrogen peroxide (0.30 mL,  $10^4$  dilution), microperoxidase (0.30 mL,  $1 \text{ mmol L}^{-1}$ ) as a catalyst and luminol (0.050 mL,  $7 \times 10^{-5} \text{ mol L}^{-1}$  in pH 9.8  $\text{Na}_2\text{CO}_3$  solution). The volumes of berry infuses were 0.05 mL. The berry samples were added to luminol solution and mixed with vortex for 10 seconds before measuring. Scavenger capacities of the samples were expressed in the relative light unit (RLU%). The RLU% was calculated from the relative light intensity and the standard background.

### Hydrogen-donating ability

Hydrogen donating ability was measured by Hatano et al.<sup>8</sup> Every 0.05 mL sample was diluted with 0.95 mL bidistilled water and 1.00 mL methanol. The samples were mixed with 0.50 mL 2,2-diphenyl-1-picrylhydrazyl free radical's methanolic solution (9 mg/100 mL). After 30 minutes, samples were measured with a Hitachi U-2000 spectrophotometer (Tokyo, Japan) at 517 nm against their blanks. The inhibition % of the samples was expressed in the percentage of the control.

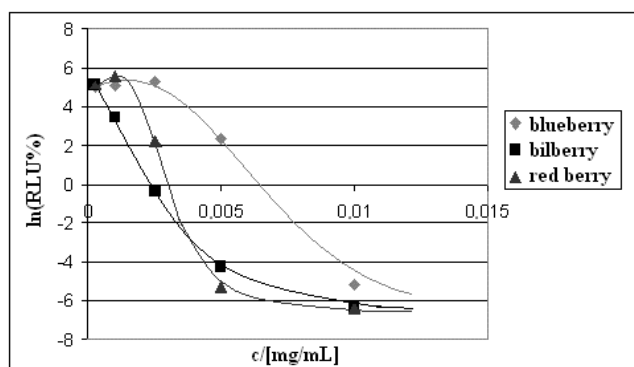
### Statistical analysis

Means and standard deviations (SD) were calculated from the results obtained. One way analysis of variance (ANOVA) was used for comparing the means of the groups by Graph PAD software version 1.14 (1990). Significance was determined as  $P < 0.05$ .

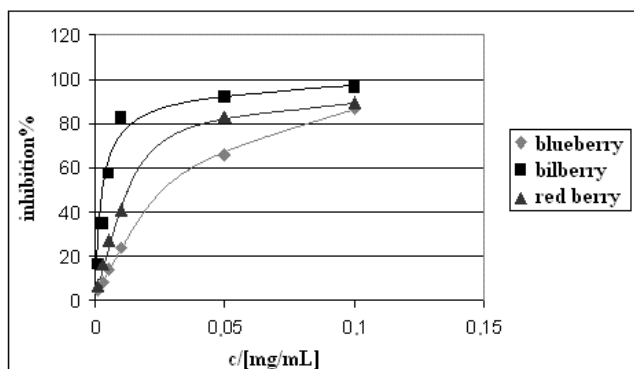
## Results

The element concentrations observed in berries are shown in Table 1. The concentrations of As, Co, Pb and V were under the detection limits therefore these elements were not shown in the table. The berries generally contain low amounts of elements similarly to the results of other berries obtained by several authors and various berries of Rubus family.<sup>9,10,11,12</sup> We noticed that almost all element concentrations were significantly different and red berry contains elements in the highest concentrations.

The antioxidant properties of the berries are shown in Figure 1. and 2. The luminol/ $\text{H}_2\text{O}_2$ / $\text{OH}\cdot$ -system emits light in alkaline solution, meaning scavengers can attenuate the emission. RLU% of red berry and bilberry is lower than that of blueberry in most of the concentration range, so their scavenging capacity is better than that of blueberry (Fig. 1.). This kind of lower antioxidant activity can be observed in the hydrogen-donating ability as well (Fig. 2.), and the difference between red berry and bilberry is higher than their chemiluminescent data. Finally after observations it was concluded that these three types of berries have good antioxidant activity.<sup>1,2,13,14</sup> Bilberry shows the highest and blueberry the weakest properties.



**Figure 1.** Free radical scavenging capacity in different berries originated from Latvia



**Figure 2.** Hydrogen-donating ability in Latvian blueberry, bilberry and red berry

## Discussion

The berries under investigations contain elements in relatively low concentration. The consumption of these kinds of fruits is also unsatisfactory. Even after consuming 100 g berry per day, the quantities of metal ions in berries do not cover the daily need. Nevertheless they may be good sources for some elements, since they contain some essential elements in higher quantity, which means 15% or higher rate of the Recommended Dietary Allowances (RDA) or Dietary Reference Intake (DRI).<sup>15,16</sup>

According to RDA parameters and taking into consideration consumption of 100 g sample of berries, the following berries are found to be good sources of: blueberry for molybdenum (37.4% of the daily need, RDA value:  $50 \mu\text{g day}^{-1}$ /adult of 70 kg); bilberry for manganese (94.1 % of the daily need, RDA value:  $2 \text{ mg day}^{-1}$ /adult of 70 kg), molybdenum (27.6 % of the daily need, RDA value:  $50 \mu\text{g day}^{-1}$ /adult of 70 kg) and red berry for chromium (15 % of the daily need, RDA value:  $40 \mu\text{g day}^{-1}$ /adult of 70 kg), manganese (101 % of the daily need, RDA value:  $2 \text{ mg day}^{-1}$ /adult of 70 kg), molybdenum (60 % of the daily need, RDA value:  $50 \mu\text{g day}^{-1}$ /adult of 70 kg). It should be also mentioned, that the Upper Level (UL) for manganese was  $11 \text{ mg day}^{-1}$ /adult earlier, nevertheless according to the EFSA,<sup>17</sup> there is not enough data available for the correct determination of this value and there is no UL value at present which would establish red berry as a safe fruit.

**Table 1.** Element concentrations (mg kg<sup>-1</sup> wet weight ± standard deviation, n=3) in different berries originated from Latvia

	Blueberry	Bilberry	Red berry	ANOVA (P<0.05)
Al	1.63 ± 0.28	3.06± 0.17	5.91± 1.08	Sign.
B	1.53 ± 0.09	1.26± 0.11	2.55 ± 0.28	Sign.
Ba	0.228 ± 0.011	1.25 ± 0.07	1.46 ± 0.04	Sign.
Ca	56.98 ± 2.35	160.4 ± 9.5	240.7 ± 16.8	Sign.
Cd	<0.01	0.013±0.017	<0.01	
Cr	0.038 ± 0.011	0.021 ± 0.003	0.060 ± 0.006	Sign.
Cu	0.728 ± 0.111	0.309 ± 0.021	0.907 ± 0.120	Sign.
Fe	2.36 ± 0.65	1.69 ± 0.06	3.25 ± 0.14	Sign.
K	387.7 ± 70.1	482.4 ± 15.5	1005 ± 48	Sign.
Li	0.00138 ± 0.00020	0.00196 ± 0.00032	0.00388 ± 0.00225	Non sign
Mg	43.72± 9.42	53.12 ± 4.28	103.2 ± 6.12	Sign.
Mn	0.684 ± 0.133	18.82 ± 0.49	20.29 ± 0.231	Sign.
Mo	0.187 ± 0.071	0.138 ± 0.029	0.309 ± 0.022	Sign.
Na	6.17 ± 0.65	6.61 ± 1.02	9.76 ± 1.00	Sign.
Ni	0.0235 ± 0.0051	0.0217 ± 0.0067	0.0387 ± 0.0059	Sign.
P	72.51 ± 9.37	95.23± 4.09	166.4 ± 6.5	Sign.
S	43.44 ± 8.07	51.03 ± 3.60	94.33 ± 4.97	Sign.
Si	8.31 ± 0.69	5.11 ± 0.45	9.57 ± 2.32	Sign.
Sn	0.482 ± 0.207	0.051 ± 0.027	0.279 ± 0.187	Sign.
Sr	0.0623 ± 0.0066	0.0631 ± 0.0041	0.159 ± 0.0095	Sign.
Ti	0.0508 ± 0.0103	0.0471 ± 0.0085	0.0691 ± 0.0096	Sign.
Zn	0.637 ± 0.059	1.16 ± 0.17	2.19 ± 0.12	Sign.

It has to be mentioned here as well that they may contain other toxic bioactive agents, like arbutin and derivatives, therefore the precaution is needed.<sup>17</sup>

The intake of non-essential elements is relatively low, instead of aluminum in the case of bilberry, which is 14.4-19.0% (DRI value between: 3.1-4.1 mg day<sup>-1</sup>/adult of 70 kg), of boron in the case of blueberry and red berry which are 15.9 and 26.6% (DRI value: 0.96 mg day<sup>-1</sup>/adult of 70 kg) and of lithium in the case of bilberry and red berry with intake of 19.6% and 38.8% (DRI value: 1 µg day<sup>-1</sup>/adult of 70 kg).

All of these three varieties of berries have good scavenging capacity and hydrogen-donating activity. The best properties are in bilberry sample, but to evaluate their difference, a wider study is needed.

These fruits have good antioxidant properties that may be associated to their valuable compounds, like flavonoids, unsaturated fatty acids and vitamins which are very important in cellular signal transduction routes to rebuild the redox homeostasis in different diseases such as in various tumors, liver and bowel diseases as well as in simple flu.<sup>1,2,4,17,18,19, 20,21,22</sup> The relatively low metal content and the high antioxidant activity suggest, that these fruits could be good supplements in metal accumulating disorders as well, like Wilson-disease, *porphyria cutanea tarda*, hemochromatosis and some metal-accumulating tumors.<sup>23</sup>

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